Fig. 1 Mass spectrum of phorateoxon sulfone

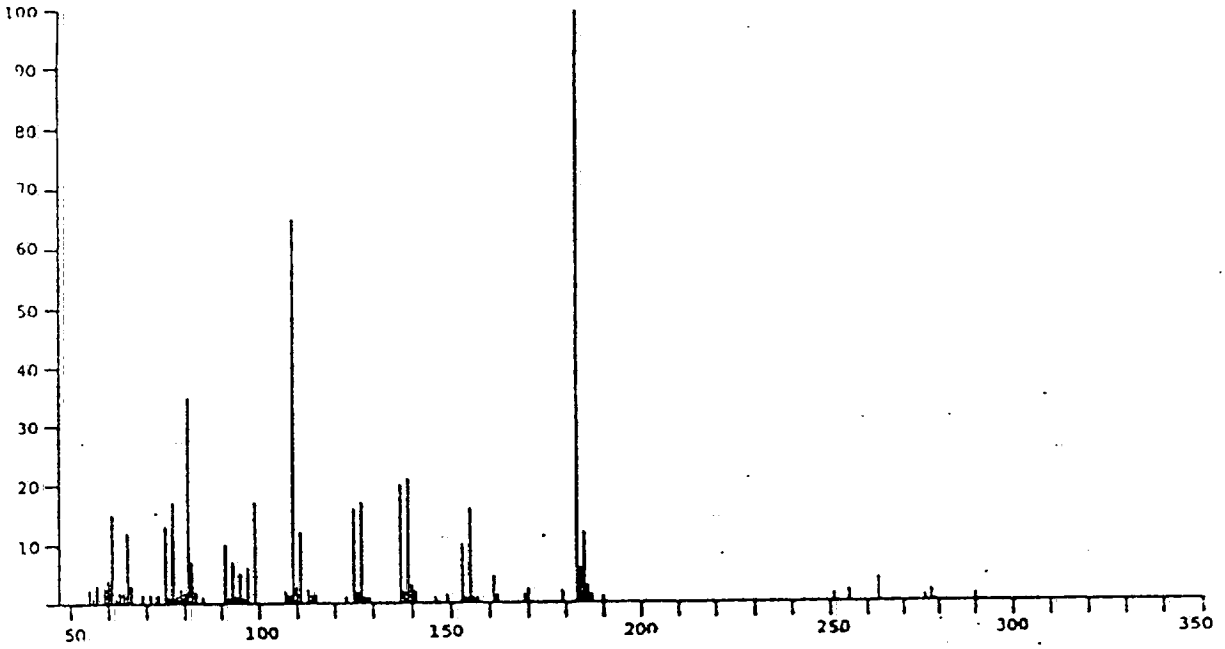
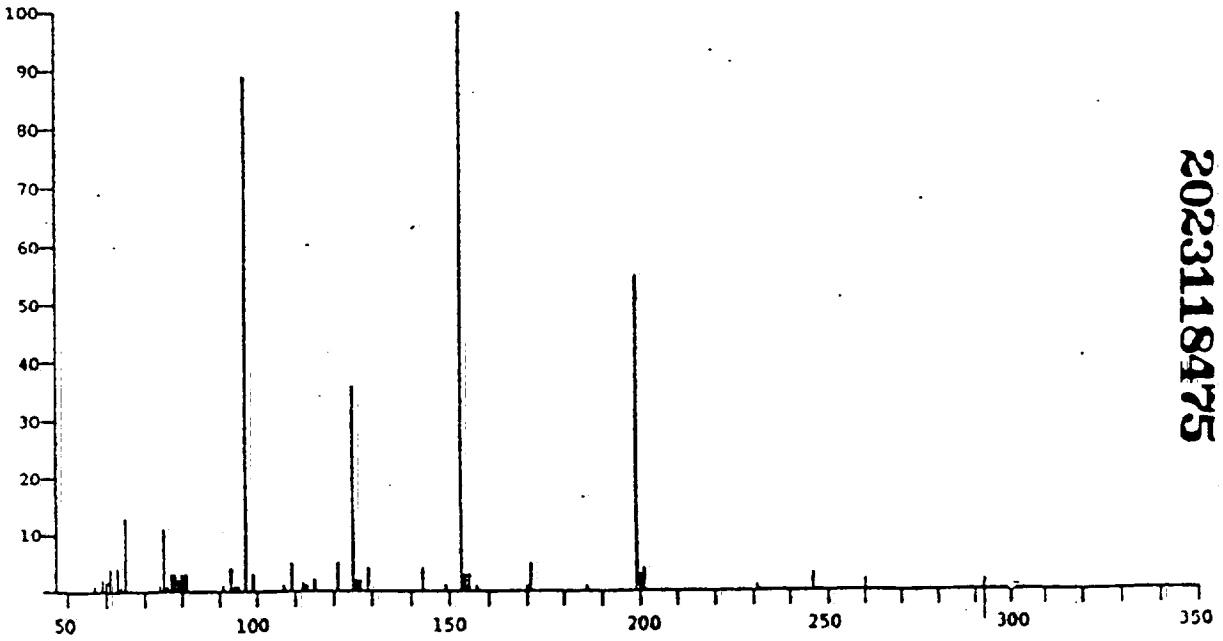


Fig. 2 Mass spectrum of phorate sulfoxide



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$KMnO_4$. All self synthesized substances were TLC purified and their identity was verified by MS (10).

(k) Test tobacco : oriental (Prilep) tobacco type.

Apparatus

(a) Ultra-Turrax (type 45).
(b) Rotatory vacuum exaporator.
(c) Basic TLC equipment.
(d) Thermostat.

Extraction

5 g dry powdered tobacco are extracted with 200 ml acetone for 5 minutes on Ultra-Turrax at moderate speed. The sample is filtered through Celite. A 50 ml aliquot is taken and vacuum evaporated to about 1 ml.

Oxidation and clean-up

To the residue in the flask 25 ml of oxidant is added. The reaction mixture is heated to about 35°C and magnetically stirred for 20 minutes. The oxidation is followed by addition of saturated aqueous solution of Na_2SO_3 until the violet colour disappears. The sample is transferred to a separating funnel, 20 ml of n-hexane are added and shaken about 1 minute. The water extract is retained. The n-hexane phase is re-extracted with 20 ml water and the water phase added to the first water extract. The oxon sulfones are extracted from water solution into dichloromethane (3X 20 ml). Finally, the dichloromethane extract is washed once with 25 ml water (for elimination of residues of acetic acid). The dichloromethane extract is filtered through Na_2SO_4 anhydrous and vacuum evaporated at room temperature to appropriate volume (for samples with unknown concentration to 10 ml).

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TLC determination

Method : Ascending, one dimensional developing in previously saturated tank. Layer : silica gel 60 G, self spreaded on 20 X 20 cm glass plate, layer thickness : 250 um immediately after spreading, used without activation, only dried over the night. Spotting : usually 25 ul extract is spotted on a plate 1,5 cm from the bottom. Four standards are always running on the same plate, dissolved in the same solvent as the sample and spotted in the same manner (five drops à 5 ul). Developing solvent : benzene-acetone 7 + 3. Height of developments : 15 cm. Time of development : about 35 minutes. Detection : dried developed plate is sprayed with enzyme solution until it is evenly wetted. Bromination is not used. The plate is incubated for 30 minutes in a chamber saturated with water vapour at 37°C. Still warm and wet, the plate is sprayed with freshly prepared solutions of 2-naphtylacetate and Fast blue salt B mixed in proportion 1:4 until the white spots on a violet background begin to appear (5 ml is enough for 1 plate).

RESULTS AND DISCUSSION

Approximate R_f values for oxon sulfones for described conditions are : 0,54 for disulfotonoxon sulfon, 0,62 for phorateoxon sulfone and 0,68 for terbufosoxon sulfone.

Two facts were basic in the developing of this relatively simple method of detection of disulfoton, phorate or terbufos toxic residues in tobacco :

1. Good selectivities and recoveries in described oxidation conditions with reference substances to the corresponding oxon sulfones.
2. Great sensitivity of Ackerman's esterase inhibition technique for the oxon sulfones.

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but their oxidative metabolites (sulfoxides and sulfones as the main, oxon sulfoxides and oxon sulfones as the minor residues) are to be looked for and determined. Because of such a great number of possible compounds, the residue analysis becomes difficult. The TLC method developed represents an approach in the residue analysis of disulfoton, phorate and terbufos in tobacco, usable for screening or confirmation purposes.

EXPERIMENTAL

Reagents

(a) Solvents : acetone, benzene, n-hexane, dichloromethane, ethanol - all p.a. (Merck).

(b) Celite 545.

(c) Oxidation reagent : 6,4 g $KMnO_4$, 50 ml acetic acid and water to 1000 ml.

(d) Na_2SO_3, saturated solution in water.

(e) Na_2SO_4 anhydrous.

(f) Silica gel 60 G (Merck 7731).

(g) Beef liver water extract as source of esterase, diluted as necessary for optimal working conditions.

(h) 2-naphtyl acetate, 1,25 mg/ml ethanol, freshly prepared.

(i) Fast blue salt B, 1,25 mg/ml water, prepared before spraying.

(j) Reference substances : phorate (98% pure), terbufos, terbufos sulfoxide, terbufos sulfone, terbufosoxon sulfoxide and terbufosoxon sulfone (all at least 90% pure) kindly supplied by Cyanamide of Great Britain Ltd. (Gosport, Hampshire), disulfoton (95% pure) Sandoz, disulfoton and phorate sulfoxides prepared from technical insecticides (active ingredient (a.i.) in Solvirex 10 and Thimet G 5) by selective oxidation with H_2O_2, disulfoton and phorate sulfones, prepared from technical a.i. by selective oxidation with neutral aqueous solution of $KMnO_4$, disulfotonoxon sulfone, phorateoxon sulfone, prepared from technical a.i. by selective oxidation with weakly acidic

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A METHOD FOR THE SEMI-QUANTITATIVE DETERMINATION OF RESIDUES OF DISULFOTON, PHORATE AND TERBUFOS IN TOBACCO

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SUMMARY

An oxidative conversion of all residues of disulfoton, phorate or terbufos to the corresponding oxon sulfones (as confirmed by mass spectrometry) was performed on an aliquot of extract of dry tobacco. Thin-layer chromatography and Ackermann's esterase inhibition technique were employed for the separation and the semi-quantitative evaluation of the residues. High recoveries for the three above-mentioned insecticides (except terbufos) and their the most important metabolites (sulfoxides and sulfones) from dry tobacco were attained. The limit of detection of total residues was 0,1 mg/kg (expressed as active ingredient in dry tobacco).

INTRODUCTION

Disulfoton, phorate and terbufos are chemically related, very active, systemic organophosphorus insecticides. The first two are known and used for many years, so many papers were published about their metabolism in plants and also methods for determination of their residues (1-8). Terbofus came in use after 1970, but a lot of results obtained for the first two insecticides seem to be directly usable for it (9). The basic principle in the analysis of residues of disulfoton, phorate and terbufos in plants is that not the active ingredients themselves

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The oxidation step in the procedure has also a considerable cleaning effect on sample, which enables application of an aliquot of the sample for TLC as big as to reach the desired limit of detection. The work is somewhat easier with terbufos and phorate residues because of low detection limits of their oxon sulfone (about 0.1 ng) comparing with the detection limit of disulfotonoxon sulfone (about 2 ng). There are more variants of TLC application of esterase inhibition technique (14-18). It is well known that the sensitivity depends on the source of enzyme and its concentration, but it seems that the way of visualisation is also important. Published detecting limits of disulfotonoxon sulfon and phorateoxon sulfone by Mendoza's esterase inhibition technique (in something different TLC conditions) are much higher (400 ng and 5 ng) (19). The basic relationship that the detection limit for disulfotonoxon sulfon is considerably higher than for the other two compounds is , however, confirmed.

The recovery experiments in presence of tobacco with three insecticides and their three oxidation products at the residue levels (0,5 mg/kg to 1 mg/kg) were satisfactory except for terbufos (only about 20% recovery). It seems that the sample consumes too much oxidant, so perhaps a greater concentration of $KMnO_4$ is needed. However, this fact does not create such a problem if one keeps in mind that terbufos itself is not expected to be present as residue in dry tobacco.

Because of the extremely great sensitivity of enzymatic detection all usually precautious as for trace analysis are necessary - careful cleaning of glassware, running of "reagent blind". Running of untreated tobacco samples is also recommended. Although the method developed here does not need much work and chemicals it is perhaps possible to perform micro analysis on a smaller aliquot of tobacco extract (practical for multi-residual and confirmating analysis), which remains to be studied.

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In searching optimum oxidation, many oxidants and conditions were tested. Until now in the most cases for the same purpose used reagent, 3-chloroperbenzoic acid (K&K Laboratories!) (6,11) was compared with proposed $KMnO_4$ in weak acid conditions. The oxidation products were analysed by TLC with $PdCl_2/J_2$ detection (12). In all cases of oxidation of microquantities of named insecticides and their oxidation products the $KMnO_4$/ acetic acid reagent was superior. The oxon sulfones were the only products with good recoveries. Also, the oxon sulfones were stable in the oxidation conditions. Increased concentrations of acetic acid in oxidation solution resulted in worse recovery of oxon sulfones - a result which has been known long ago for the oxidation of phorate (11). With neutral solution of $KMnO_4$ (0,1 or 0,5 N) the P=S compounds give sulfones (and oxon sulfones in traces) and only P=O compounds oxon sulfones as oxidation products. This reaction is usually used in the methods for the GLC determination of total residues of this group of insecticides (1,3,7). Although the $PdCl_2/J_2$ detection makes possible to differentiate between sulfoxides and oxon sulfones in doubtful TLC separation (because of different colours of the spots), the MS identification of oxon sulfones was also performed, (9,13,14). The molecular masses of corresponding sulfoxides and oxon sulfones are equal and the molecular peaks in our conditions of taking off the mass spectra of low resolution (mass spectrometer CEC 21-110 B, ionisation energy : 70 eV, source temperature : 60-100°C, direct insertion of compounds) were weak for both types of compounds, but the differences in MS are obvious and well usable for the identification : the most intensive and at the same time characteristic peaks arise from fragments after delta cleavage of the disulfoton derivate with regard to phosphorous atom and further elimination of two ethen molecules (m/e 197, 169 and 141 in MS of disulfotonoxon sulfon). This corresponds to gamma cleavage in phorate and terbufos oxon sulfones (m/e 193, 155 and 127). Peaks in common for all three MS, give alpha cleavage (m/e 137, 109 and 81) Characteristical for the MS of all terbufos derivatives is the peak at m/e 57 (t-Bu, usually the base peak), which is the main difference to the MS of phorate derivatives.

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